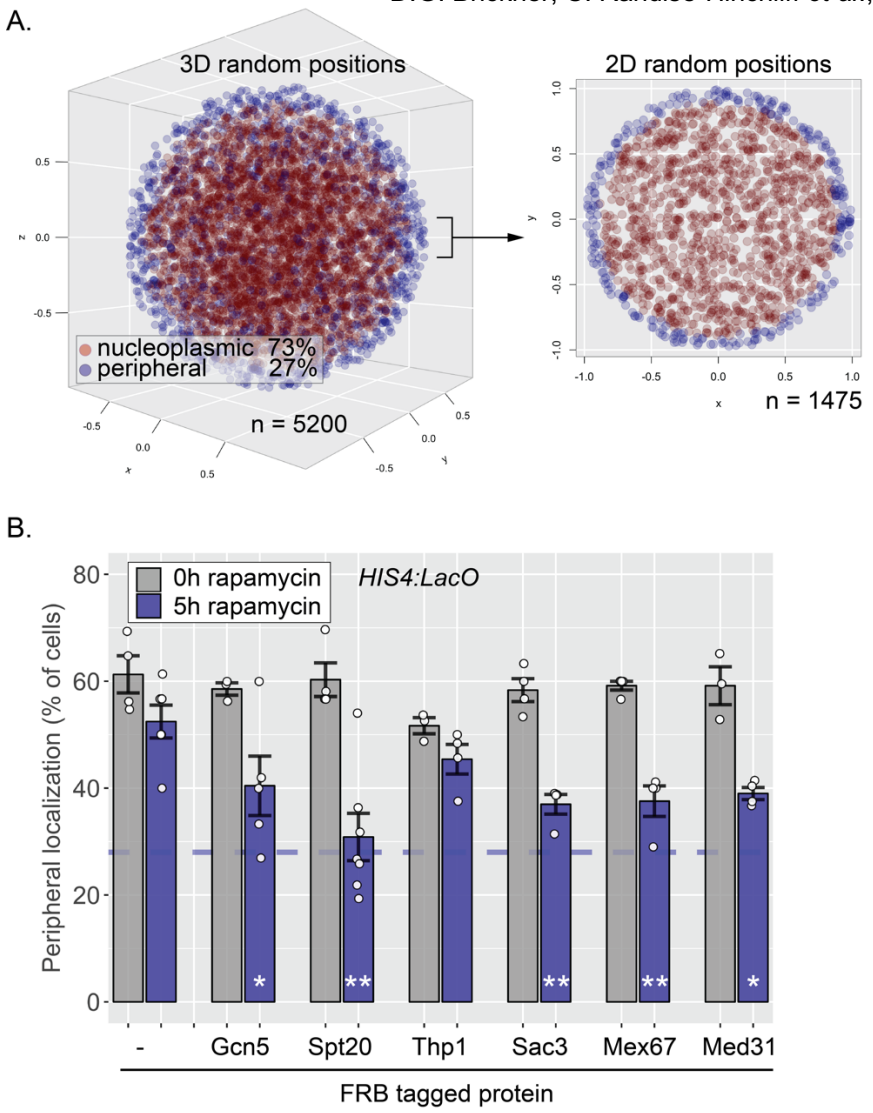
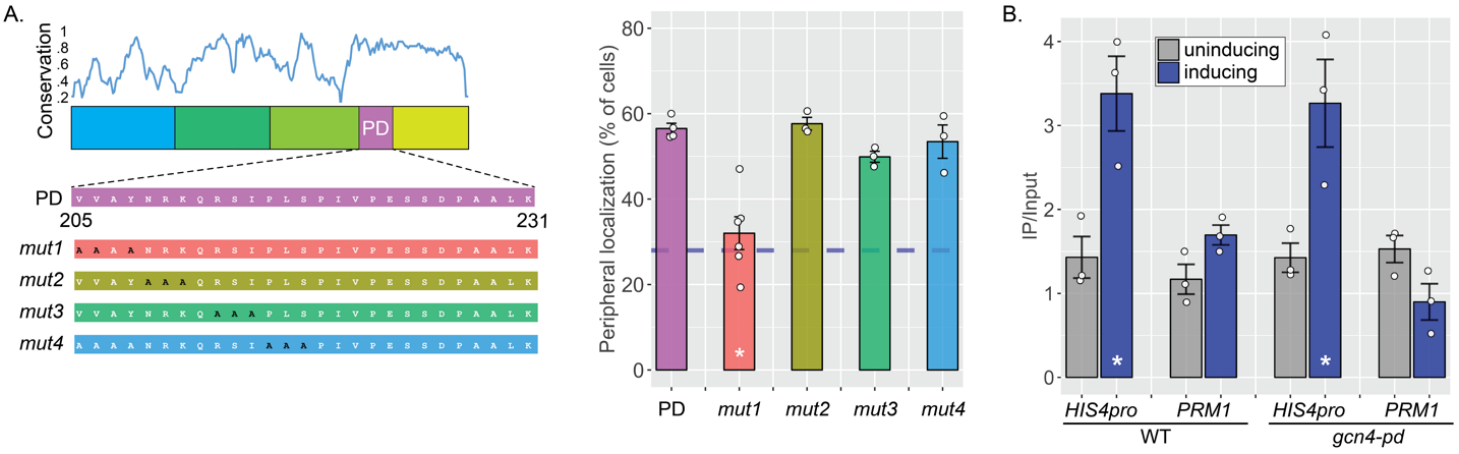


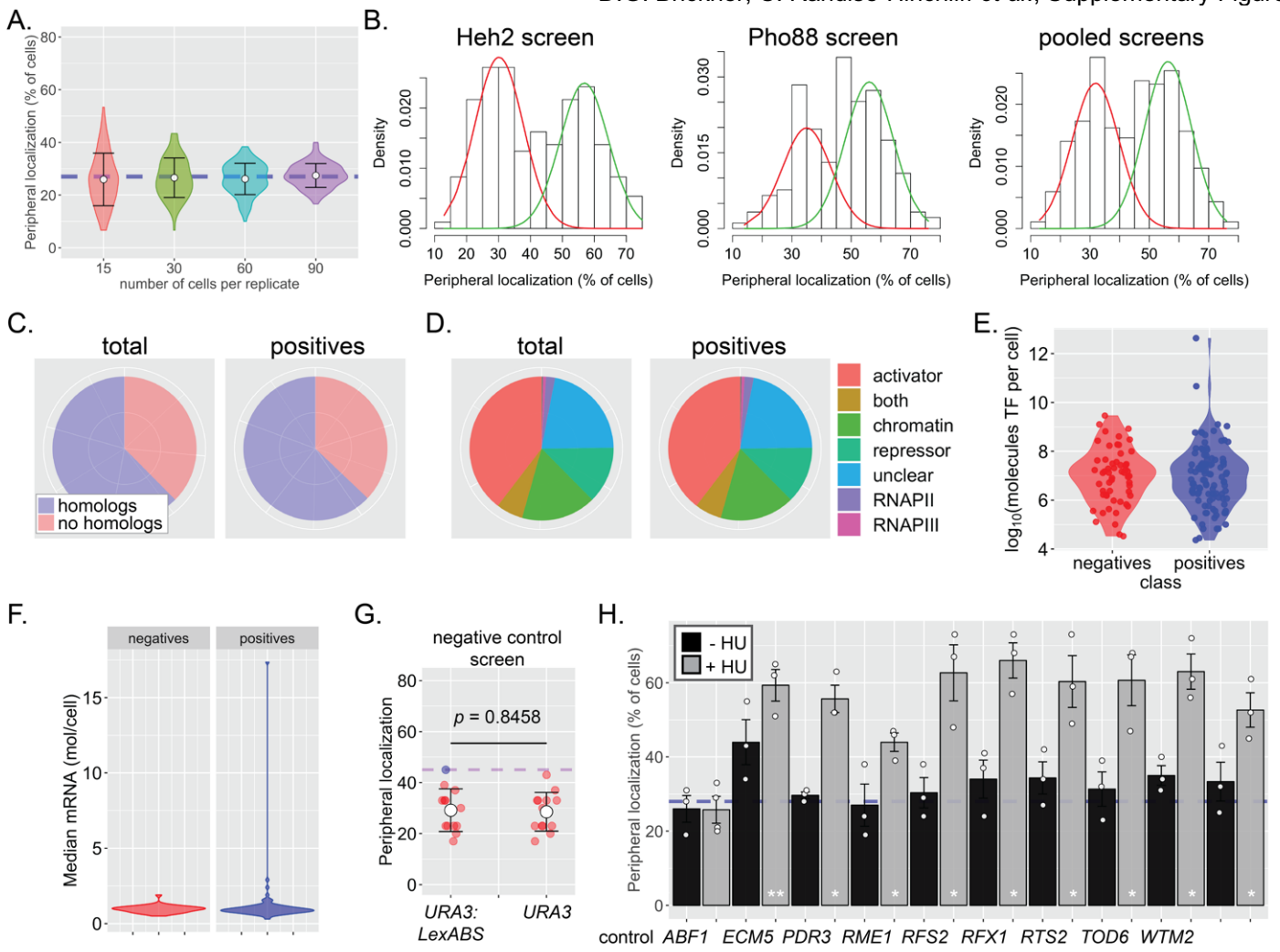
Brickner et al., Figure S1, related to Figure 1

Figure S1, related to Figure 1. Simulation of localization and effect of long-term Anchor Away on *HIS4* localization. A. 5,200 simulated random positions within a sphere of radius = 1μm. Left: 3-dimensional plot. Right: 2-dimensional plot of the equatorial slice from the sphere (± 0.2μm from the origin). The subset of positions that are ≤ 0.1μm of the edge (i.e. distance to the center ≥ 0.9μm) are blue, while those that are nucleoplasmic (*i.e.* distance to the center < 0.9μm) are red; fraction of total in each class is indicated. **B.** Localization of *HIS4-LacO* upon Anchor-Away of each of the indicated factors for 5h (-, no FRB control). Student's t-test to compare with the 0-minute time point; * $p < 0.05$; ** $p < 0.01$.



Brickner et al., Figure S2, related to Figure 2

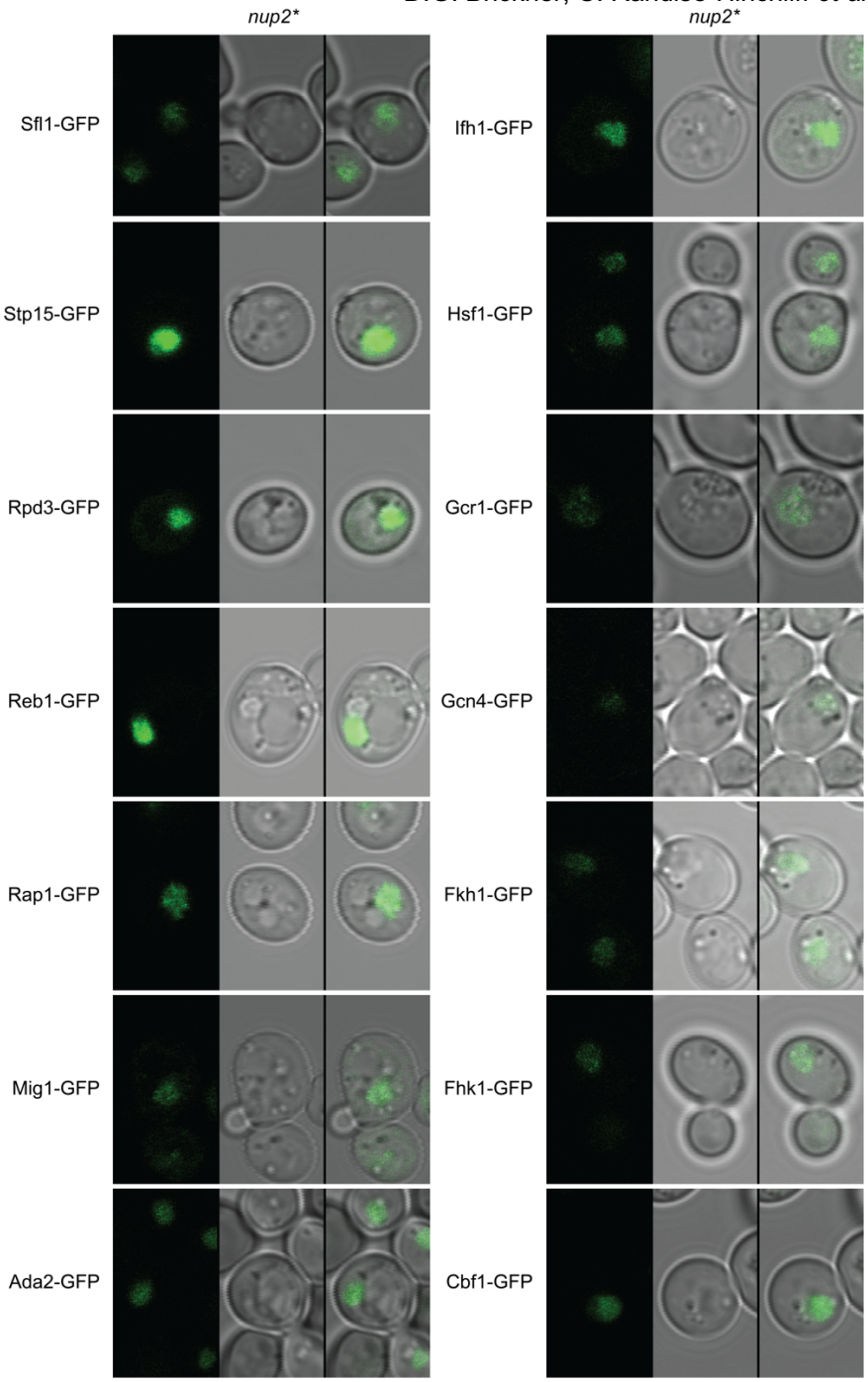
Figure S2, related to Figure 2. Identification of the *pdmut*. A. Left: schematic of mutations introduced into the PD_{GCN4} highlighted in black. Right: LexA fusions of each protein were expressed and scored for peripheral localization from ≥ 3 biological replicates of ≥ 30 cells each. Data for each replicate are indicated with white circles and the mean and SEM are plotted. **B.** ChIP against myc-tagged Gcn4 or Gcn4-pd from cells grown in either uninducing (+histidine) or inducing (-histidine) conditions. The recovery of the *HIS4* promoter or the *PRM1* coding sequence was quantified by real-time quantitative PCR. Student's t-test to compare to the wild type PD-LexA (A) or to the uninducing condition (B); * $p < 0.05$.



Brickner et al., Figure S3, related to Figure 3

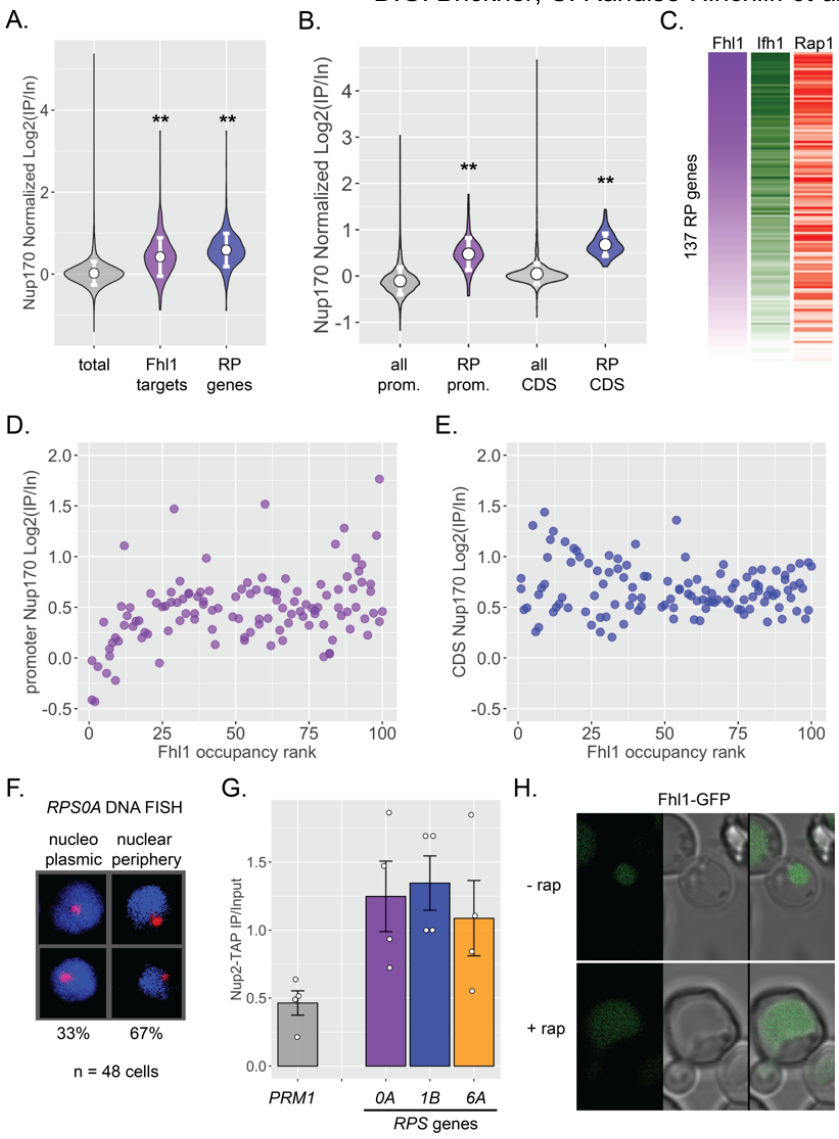
Figure S3, related to Figure 3. Tethering screen simulations and results. A. From the simulation in Figure S1, 100 samples of 15, 30, 60 or 90 cells were generated. Shown is the overall distribution of these samples, as well as their mean (white circle) and the standard deviation. The true mean of the simulated population (n = 5200) is indicated with the blue, hatched line. **B.** The distribution of percent peripheral from each of the screens, as well as the pooled result was modeled as a bimodal distribution using the R package *mixtools*, version 1.1.0. The resulting models, derived from iterative fitting, are shown. **C.** The fraction of the TFs for which there are clear metazoan homologs from either the entire set or from the positives. **D.** Classification of the TFs in the entire set or in the positives. **E.** The abundance of the TFs, categorized according to peripheral localization. **F.** The median abundance of the mRNAs of the targets of TFs, categorized as either negatives or positives from our screens. **G.** A collection of 13 enzymes and non-DNA

binding proteins tagged with the LexA DBD and crossed against the indicated strains. The mean and standard deviation for the set of strains is also shown. **H.** Peripheral localization of *URA3:LexABS* in strains having the indicated TFs tagged with LexA DNA binding domain grown in the presence or absence of hydroxyurea (1h treatment). Student's t-test was performed to compare to control under the same condition; * $p < 0.05$; ** $p < 0.01$.



Brickner et al., Figure S4, related to Figure 4

Figure S4, related to Figure 4. Inactivation of Nup2 has no effect on nuclear localization of select TFs. Nup2 was inactivated in strains expressing each of the indicated GFP-tagged TFs using CRISPR/Cas9. Each strain was imaged using a Leica SP8 confocal microscope.



Brickner et al., Figure S5, related to Figure 5

Figure S5, related to Figure 5. Validation of Fhl1/Ifh1 target genes. A. Distribution of normalized Nup170 ChIP against all probes, Fhl1 target genes or 137 ribosomal protein genes (Van de Vosse et al., 2013). **B.** Distribution of Nup170 ChIP over promoters and coding sequences for all genes and for ribosomal protein genes. **C.** The rank order of Fhl1, Ifh1 and Rap1 occupancy over 137 ribosomal protein genes (from Reja et al., 2015) used in panels D & E. D. Rank of Fhl1 occupancy vs. Nup170 promoter occupancy. **E.** Rank of Fhl1 occupancy vs. Nup170 coding sequence occupancy. **F.** DNA FISH against RPS0A. **G.** ChIP for Nup2-TAP (Ghaemmaghami et al., 2003), quantifying recovery of *RPS0A*, *RPS1B* and *RPS6A* promoters. **H.** Anchor away strain expressing GFP-FRB tagged Fhl1 was imaged before and after treatment with rapamycin for 1h.